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(54) **Method and apparatus for electrochemical measurements.**

(57) A method for measurement or detection of a component of an aqueous liquid sample, said method comprising:
placing a sample, possibly containing the component of interest, into a reaction cell, said reaction cell comprising at least two electrodes having an imedance which renders them suitable for coulometric, amperometric or voltammetric processes, so that the sample forms a layer of liquid having a thickness less than about 0.2 millimeter in a reaction zone overlying one of said electrodes,
reacting said component, if present, directly or indirectly with a redox reagent represented by one of said electrodes to cause deposition of a derivative of said component on said one electrode, and monitoring current on deposition or subsequent stripping of said derivative to provide an indication of the presence or quantity of said component of interest by coulometry, voltammetry or amperometry.

EP 0 470 649 A2

Field of the Invention

This invention relates to methods and apparatus for making electrochemical measurements, in particular but not exclusively for the purpose of carrying out microchemical testing on small liquid samples of biological, e.g. clinical origin.

Background to the Invention

F. Schlapfer et al (Clin.Chim.Acta, 1974, pp 283-289) described electrochemical measurement of glucose concentration using glucose oxidase and soluble electron transfer substances such as ferricyanide, p-benzoquinone, 2,6-dichlorophenolindophenol, pyocyanine, thionine or methylene blue, interacting with amperometric noble-metal electrodes.

These arrangements have not given rise to glucose-measurement products which are simple and convenient to use in environments far removed from the skilled inhabitants of analytical laboratories.

Since 1974 a variety of further electrode arrangements have been proposed for chemical/immunochemical analysis, among them electrodes carrying immobilised redox mediators as well as enzymes, for example EP 0078636, 0125136 and 0125139 (Genetics International), and 0142301 (Serono). EP 0177743 (Shimadzu) describes enzyme electrodes of somewhat complex construction, which are capable of use to measure a number of enzyme substrates by amperometry, using electron transfer mediators.

EP 0125137 discloses an electrode sensing system, for monitoring components in a liquid mixture, using a probe-type sensor which typically comprises an electrode surface carrying a generally insoluble electron transfer mediator, in turn coated with an enzyme, the electron transfer mediator acting to transfer charge between the enzyme and the electrode. The system is typically used to determine glucose concentrations, using amperometric techniques.

The paper in "Analytical Chemistry", Volume 56, No 2, February 1984, pages 148 to 152 discloses an enzyme electrode using a thin layer platinum electrode in conjunction with the enzyme glucose oxidase or flavine adenine dinucleotide, and refers to the possibility of reducing the depth of the reaction chamber to optimize performance.

This invention aims to provide measurement apparatus and methods to enable quick, convenient and accurate measurement of various constituents of liquid samples, especially of biological origin, e.g. clinical samples of blood, serum, or urine.

The invention also aims to provide measurement apparatus of simple construction which can be treated as disposables.

It is a further aim of the present invention to provide electrochemical cells for convenient liquid sample analysis but without requiring complex electrode structures involving immobilised components.

Summary of the invention

According to one aspect of the invention there is provided a method for measurement or detection of a component of an aqueous liquid sample, said method comprising:

placing a sample, possibly containing the component of interest, into a reaction cell, said reaction cell comprising at least two electrodes having an impedance which renders them suitable for coulometric, amperometric or voltammetric processes, so that the sample forms a layer of liquid having a thickness less than about 0.2 millimeter in a reaction zone overlying one of said electrodes,

reacting said component, if present, directly or indirectly with a redox reagent represented by one of said electrodes to cause deposition of a derivative of said component on said one electrode, and monitoring current on deposition or subsequent stripping of said derivative to provide an indication of the presence or quantity of said component of interest by coulometry, voltammetry or amperometry.

The result relating to the quantity of the substance to be measured can be of use, among other things, as an index of the concentration of the substance in a liquid sample.

In this specifiction and claims 'redox reagent' and similar terms and 'electron transfer reagent' and corresponding terms are mutually inclusive.

The invention also encompasses apparatus for carrying out electrochemical detection or measurement of a component of an aqueous liquid sample, said apparatus comprising:

a reaction cell comprising at least two electrodes, the electrodes having an impedance which renders them suitable for coulometric, amperometric or voltammetric processes and being located in or adjacent to a reaction zone, said reaction zone being capable of receiving an aqueous liquid sample possibly containing said component of interest, and said electrodes and said reaction zone being arranged so that liquid in said zone contacts said electrodes and forms a layer having a thickness less than about 0.2 millimeter overlying one of said electrodes,

and said cell comprising a redox reagent represented by one of said electrodes so that a derivative of said component is deposited on said one electrode on application of a suitable electrical potential.

It is especially preferred to provide in the use of this method a cell which confines the liquid reagents to react with the electrode to a sufficiently thin layer overlying the electrode to permit coulometric measurement of the electro-active material to take place in a short time. A suitable thickness for the liquid layer is for example of the order of about 0.02 to 0.2 mm, for example about 0.1 mm. Capillary-fill cells with a configuration as described in EP 0170375 (Unilever) are among the cells suitable in this respect. In certain useful arrangements within the scope of the invention, the cell may confine a defined reactive volume of sample or reaction liquid in a space of defined width between a cell wall and an electrode of defined area. Liquid outside the volume may be able to diffuse inwards but for example only at an inappreciable rate compared to the time required for reaction of the liquid in the defined volume. In other useful embodiments, the cell may define a volume of liquid to provide material to react at the electrode.

The component to be measured can in general be formed by initial enzymic or chemical conversion of any analyte: e.g. an analyte can be sucrose, and invertase can be contained in said cell to form from said sucrose glucose by hydrolysis: the glucose so formed can then be measured by the methods described herein.

An oxidoreductase enzyme can be present in said cell to mediate any desired reaction between the component to be measured and any additional electron transfer reagent.

In one class of tests which can be carried out using the devices and methods described in more detail herein, the component to be measured comprises an electrochemically reducible metal ion or an electrochemically oxidisable inorganic ion or an electrochemically oxidisable or reducible organic compound, and is measured either by direct coulometry or voltammetry or by coulometry or voltammetry after initial electrochemical conversion to an electrochemically oxidisable or reducible intermediate. In certain cases, the tests can be carried out as amperometric tests, i.e. it may not in all cases be necessary to deplete completely the electroactive species to be measured.

The form of the reaction cell in which these reactions are allowed to take place can contribute significantly to the convenience of the test procedure. It is preferred to use an adapted form of the capillary fill cells provided with electrodes as described in European Specification No 0 170 375 (Unilever), containing electrodes of suitable impedance carried as thin films on one or more walls thereof. The drawings and description of said specification are incorporated herein by reference, to be modified by the indications given herein for making and using the measurement devices and methods of the present invention.

Such a cell as adapted for the purposes of the present invention can suitably for example comprise three electrodes, viz (a) a working electrode, for example of gold or other noble metal, carbon or graphite in any convenient form, e.g. wax-impregnated graphite; (b) a counterelectrode, chosen from a similar range of materials as given for electrode (a), and possibly of the same material as electrode (a) itself; and (c) a reference electrode, for example a silver/chloride electrode, or pH electrode.

Choice of electrode materials can for many purposes preferably be made among gold, silver and carbon film electrodes. Examples of preferred uses are: gold electrodes are very suitable for voltammetry of trace quantities of mercury, and gold electrodes in combination with a thin mercury layer, e.g. produced by preliminary electrochemical deposition from a quantity of mercuric salt, are very suitable for analysis of heavy metals or organic materials such as drugs, e.g. morphine. Carbon electrodes are very suitable e.g. for electroanalysis of oxidisable organic groups such as amines, or reducible organic groups such as nitrobenzenes. The aim is to provide electrodes with low enough impedance for amperometric or voltammetric use, in a manner well known in itself.

In one convenient form, both or all electrodes are contained as films in a capillary fill cell formed between two parallel flat plates spaced apart by about 0.1 mm cell thickness, with about 0.1 mm thick tracks of sealing material forming the remaining sides of the cell apart from an aperture for entry of liquids.

It can be convenient to form such a cell using opposed plates of for example ceramic, plastics or glass. When such a cell is fabricated, as is preferred, by the use of ceramics, a suitable substrate can be for example a 96% alumina substrate (Kyocera A4476 - Trade Mark), and a preferred material for the electrodes to be formed thereon is gold, applied as gold printing paste (Engelhard T4474 - Trade Mark), to be applied in a high temperature oxidative furnace in accordance with the ordinary methods of use of that material. This results in the context of this invention in a gold layer with overlying thin oxide layer capable of constituting a highly reproducible electrode. Whenever desired, part of the metal layer can be blanked off by overlying dielectric layers e.g. formed of dielectric printing ink (DuPont 5704 - Trade Mark) applied to the substrate according to the ordinary manner of use of that material.

When reactions of the kinds described above are allowed to occur in a cell as described above, it is found that a working electrode can easily deplete

substantially all of the electroactive material in that part of the liquid that overlies the working electrode, before any substantial lateral diffusion has taken place.

Accordingly, it is preferred to use cells of such dimensions that this situation prevails: i.e. that the time required for lateral diffusion of an appreciable amount of reactive material from the region outside that which overlies the working electrode, to the region overlying the working electrode, is much longer than the time required for diffusion of cell contents across the thickness of the cell and for depletion by an electrode of the material capable of reacting with it from the region of the cell overlying said electrode.

An advantage arising from use of the invention in this manner is that calibration of the measurements can be particularly simple and uniform as between samples of the devices as described herein.

The arrangements of the invention can for example take the form of coulometric measurements or of voltammetric measurement, or of amperometric measurements. Such measurement methods are in themselves known and their details do not constitute the present invention. In certain embodiments, the process of measurement can take the form of modifications of the amperometric methods described for example in EP 0177743 (Shimadzu).

Further details of the invention are given below in connection with the following illustrative examples.

Example 1

Examples of glucose measurement will now be described non-limitatively, first in a coulometric embodiment.

Reagents for the test can conveniently be dried down on to a surface which either forms part, or will form part, of a glass or ceramic inner surface of a capillary fill cell.

The reagents can be dried down either by filling reagent liquid into a pre-formed cell (e.g. 0.1 mm wide) and then drying, or by screen-printing a liquid layer up to 0.1 mm thick to be dried on to said surface which will form part of said cell when said cell is fabricated from a component carrying dried printed reagents.

In the present example the reagents are chosen so that upon rehydration in the sample liquid filling the cell they give:-

buffer (preferably about 0.1M ammonium citrate, otherwise e.g. 0.5M sodium phosphate) adjusted to approximtely neutral pH;

0.5M potassium ferricyanide; and

0.5 mg/ml glucose oxidase (a considerable excess, which may be reduced).

Low molecular weight (about 40,000) polyvinylpyrrolidone can be used as a carrier and/or stabiliser, used in a quantity and concentration dictated largely by the volume of reagent liquid to be applied and dried, and by the method of application, e.g. at 5% w/v in liquid to be filled and dried in a preformed cell, and at higher concentration (optionally lower volume) in liquid to be printed. In other variants, any other reagents can also be present to suit the test to the test sample liquids to be used - e.g. further anticoagulant besides citrate, if necessary, where whole blood is to be tested. This example gives a sensitivity range of about 0 to 20 mmolar glucose concentration. In other variant examples, suitable concentrations for the ferricyanide lie in the order of about 3 times the maximum concentration of glucose to be estimated. Chloride ion should be present where a chloride electrode is used as the reference electrode. Also usefully present in certain variants can be an inhibitor of catalase, e.g. sodium azide, and/or a chemical deoxygenator.

The dried reagents may be carried and/or stabilised on a surface by inclusion of a water-soluble polymer, e.g. polyvinylpyrrolidone, or alternatively a water-insoluble polymer support such as a thin layer of cellulose acetate.

Test liquid is introduced into the cell. The immobilised reagents including the electron transfer substance (ferricyanide) are allowed to dissolve and disperse throughout the volume of the test liquid, and the reaction of the glucose and the glucose oxidase is allowed to take place, reducing the ferricyanide to ferrocyanide.

The electrical arrangements can comprise for example a conventional potentiostatic control arrangement in which, for example, a voltage-follower impedance transformer is connected with its input taken from the working electrode and reference electrode, and its output taken, through circuitry to apply a working low-impedance voltage between the working electrode and the counterelectrode, in a negative feedback arrangement such that the p.d. between the working electrode and reference electrode is kept close to a desired level.

A current integrator is connected with its inputs taken from the working electrode and counterelectrode, and delivers as its output a signal which is to be taken as the coulometric measurement given by the device.

Initially, the potentiostatic control is set so that the p.d. between working and reference electrode is insufficient to allow electrode reaction of the form of electron transfer substance produced indirectly by reaction of the analyte: at a point in time from which the coulometric integration is to be started, the voltage is stepped to a level that does allow

such electrode reaction. Typically, a potential at the working electrode is chosen that oversteps the redox potential of the electron transfer material by of the order of about 0.05 - 0.1 volt, to maximise the wanted reaction relative to any side reactions. Then the current is monitored and for example integrated for a wanted appropriate interval of time to provide the desired signal indicative of the wanted measurement. Suitable 'inactive' and 'active' potentials for the ferro/ferricyanide embodiments can be for example in the range up to about + 0.25 volt and + 0.5 volt respectively.

A preferred configuration for this and other examples involves the use of a cell comprising a pair of gold electrodes. In this case one gold electrode can serve as a counter-electrode as well as a reference electrode, and a substantially invariant potential can be obtained via the ferricyamide present in the reaction mixture, of which the quantity can most suitably be large, (e.g. much larger than the quantity of analyte and ferrocyamide formed by reduction,) hence substantially constant. A preferred operating potential can be at about + 0.15 volt.

It can be especially convenient to provide a simple combination of potentiostat and digital meter readout for the integrator. Then the user can watch until the digital reading comes substantially to a standstill (i.e. upon completion of the electrode reaction) and takes the reading at that point as the wanted measurement result, or in another arrangement, involving automatic data processing, the digital signal can be stored when its rate of change has subsided below a preset threshold rate.

It is found that typical electrode currents in this coulometry are of the order of fractions of a milliamp for a few minutes where typical blood glucose concentrations are measured, e.g. in whole blood, plasma or serum.

In one alternative variant of this embodiment, there may be no 'inactive' potential applied to the cell formed by the working and counter electrodes, but rather this cell may be left open-circuit until the current integration is to take place.

Example 2

Glucose measurements can be made by chronopotentiometry, using arrangements similar to those described in Example 1 above in respect of the cell, electrodes, and cell contents, but the electronic arrangements are as follows.

A high impedance measuring and indicating arrangement is used to measure the p.d. from working to reference electrode, and then a constant current, e.g. up to about 0.1 milliamp, is applied between the working and counter electrodes. The potential between working and reference electrodes thereupon starts from zero, rises to a substantially steady (in practice, slowly-rising) value for a period during which the ferrocyanide is being oxidised at the electrode. The time during which the voltage remains substantially steady or only slowly rising can be taken in combination with the current level to yield an integrated value with the same significance as before.

It can be convenient to use simple digital circuit modules to start the timer when the voltage has reached within about 5% of its steady state value, or is changing at a correspondingly slow rate, and to stop the timer on reaching a second threshold of voltage or rate of change of voltage.

In connection with the example of glucose measurement, alternative electrochemical reaction paths besides the glucose-oxidase-ferricyanide path may be used. Glucose oxidase is much the preferred material for reaction with glucose, and it can be used in combination with other electron accepts than ferricyanide, e.g. methylene blue.

Methylene blue offers the advantage over ferricyanide in being less susceptible to interfering side reactions from any ascorbic acid there may be in the test sample.

Example 3

The process of the invention can be used for trace metal analysis by anodic stripping voltammetry. In this arrangement, it can be convenient to use a noble metal or (preferably) carbon working electrode, pH buffer, and excess mercuric nitrate. In a calibration test, for example, the test liquid in the cell can contain 1 microgram/ml each of cadmium, lead and copper ions, and 20 microgram/ml mercuric ions, in acetate buffer 0.1M pH 4.8. The measurement method is as follows. After introducing the reagents into a capillary fill cell provided with electrodes as described above, (a) first a high negative potential (-1.0 volt) can be applied to the working electrode for a precisely standardised time in this instance chosen as 30 seconds, to deposit Hg metal thereon, to form a thin mercury film including as amalgam whatever traces of metals may deposit together with the mercury. When a capillary fill cell is used, complete deposition may be achieved and standardisation of time may be rendered unnecessary.

Thereafter, (b) a positive going potential ramp is applied to the working electrode, (e.g. from -1.0 volt to +0.1 volt using a differential pulse technique at an overall scan rate of 10 mV per second with +50 mV pulse height using a PAR 264 polarograph (Trade Mark) from Princeton Applied Research). Measurement of the resulting current is made to determine the occurrence of any peaks. Any peaks that occur indicate the presence of a

metal identifiable in principle by the potential at which the peak is observed, in a quantity corresponding to the area under or height of the current peak.

The reagents can be dried down as with other embodiments, and deoxygenating reagents such as ascorbic acid and/or sodium sulphite can be included. Alternatively an a.c. polarographic technique can be used with no need for the salts to be added.

Further variants of the processes are represented for example as follows:

(a) Direct voltammetry of mercuric ions using a gold electrode can be carried out by introducing a sample possibly containing a trace quantity of mercuric ions into a capillary fill cell, and imposing a negative-going potential gradient on one of the electrodes e.g. from +0.5 volt to -0.5 volt, at 5 mV/sec as a linear ramp. This produces a peak current response where the mercuric ion is reduced. The integrated quantity of the charge passed during the peak is detected or estimated as an index of the amount of the mercury present.

Alternatively the mercury can be estimated by step coulometry, which comprises holding the electrode potential more positive than the mercuric reduction potential, e.g. +0.5 volt, until other current-generating processes have decayed. Then the voltage is stepped to a value more negative than the mercury reduction potential, e.g. to -0.1 volt. Then the charge passed until the current decays can be taken as an index of the amount of mercury present.

It is of course possible to carry out variants of each of these processes in which stripping voltammetry is used, as in Example 3.

(b) Nickel can be estimated using a cell containing dimethylgyloxime as follows: In the cell there is provided as a reagent layer (together with mercuric acetate or nitrate as in Example 3) a layer of dimethylglyoxime salt, pH7. This reacts with nickel (2+) introduced in a sample to form a complex. At about -0.4 volt mercury deposits on the (e.g. gold or carbon) electrodes with an oxidised form of the nickel complex. The complex can then be stripped and analysed using a negatively going potential gradient.

In this variant, preferred electrode systems are either a 3-electrode system comprising two gold electrodes and a silver reference electrode, or a 2-electrode system comprising a gold electrode and a silver (chloride) sacrificial electrode.

(c) Organic materials, e.g. drugs such as morphine, can be estimated by absorptive stripping voltammetry using cell arrangements as described herein: holding a gold or carbon electrode at a negative potential for about a minute, and by holding the electrode at about 0 volt for about a minute the morphine may be absorbed: on reducing the electrode potential past about -0.2 volts the morphine derivative is stripped off from the working electrode and generates a current peak from the amount of which the result of the test may be obtained.

In further embodiments, it can be sufficient to use a working electrode and a further electrode combining the functions of reference and counter electrode, provided that the further electrode is a metal/metal-halide reference electrode and the corresponding halide is present in the sample, preferably at standardised high concentration. Alternatively, any other further electrode of low electrochemical impedance and adequately-defined potential may be used. Suitable electrodes for use are for example as described in EP 0186286 (Unilever).

The invention described herein is susceptible of many modifications and variations as will be apparent to the skilled reader, and the disclosure herein extend to the use of all combinations and subcombinations of the features and characteristics described, shown and/or mentioned herein and in the drawings referred to in a specification incorporated by reference.

Claims

1. A method for measurement or detection of a component of an aqueous liquid sample, said method comprising:

   placing a sample, possibly containing the component of interest, into a reaction cell, said reaction cell comprising at least two electrodes having an imedance which renders them suitable for coulometric, amperometric or voltammetric processes, so that the sample forms a layer of liquid having a thickness less than about 0.2 millimeter in a reaction zone overlying one of said electrodes,

   reacting said component, if present, directly or indirectly with a redox reagent represented by one of said electrodes to cause deposition of a derivative of said component on said one electrode, and monitoring current on deposition or subsequent stripping of said derivative to provide an indication of the presence or quantity of said component of interest by coulometry, voltammetry or amperometry.

2. A method according to claim 1, wherein said component to be measured comprises an electrochemically reducible ion, an electrochemically oxidizable inorganic ion or an electro-

chemically oxidizable or reducible organic compound.

3. A method according to claim 1 or 2, wherein a trace metal is analysed by anodic stripping voltammetry.

4. A method according to claim 1 or 2, wherein mercuric ions are analysed by direct voltammetry or by step coulometry.

5. A method according to claim 1 or 2, wherein organic materials are estimated by absorptive stripping voltammetry.

6. A method according to any one of claims 1 to 5, wherein said reaction cell comprises a capillary fill device.

7. Apparatus for carrying out electrochemical detection or measurement of a component of an aqueous liquid sample, said apparatus comprising:

a reaction cell comprising at least two electrodes, the electrodes having an impedance which renders them suitable for coulometric, amperometric or voltammetric processes and being located in or adjacent to a reaction zone, said reaction zone being capable of receiving an aqueous liquid sample possible containing said component of interest, and said electrodes and said reaction zone being arranged so that liquid in said zone contacts said electrodes and forms a layer having a thickness less than about 0.2 millimeter overlying one of said electrodes,

and said cell comprising a redox reagent represented by one of said electrodes so that derivative of said component is deposited on said one electrode on application of a suitable electrical potential.

8. Apparatus according to claim 7, wherein said electrodes are selected from the group comprising gold, carbon and silver electrodes.

9. Apparatus according to claim 7 or 8 wherein said reaction cell comprises a capillary fill device.